Chromatographic Studies of 9-Acridine Derivatives

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Abstract □ GC and thin-layer fluorescent scanning studies are described for the analysis of 9-alkylaminoacridines and 9-alkylacridine derivatives. Procedures were developed for the analysis of 9-alkylaminoacridines in the presence of their hydrolytic products. Quinacrine in the presence of 6-chloro-2-methoxyacridone and 6-chloro-2-methoxy-9-aminoacridine served as a model system in these studies.

Keyphrases □ 9-Acridine derivatives—GLC and TLC (fluorescent scanning) studies, analysis of 9-alkylaminoacridines in presence of their hydrolytic products □ 9-Alkylaminoacridines—analysis in presence of their hydrolytic products □ GLC—analysis of 9-acridine derivatives □ TLC—fluorescent scanning analysis of 9-acridine derivatives

There is interest in these laboratories in the synthesis of both 9-alkylaminoacridine and 9-alkylacridine derivatives as potential fluorescent labeling agents. Analytical procedures were developed in support of such studies to examine the purity of these and related compounds. Of particular interest were procedures for the determination of the purity of 9-alkylaminoacridines in the presence of their potential hydrolytic products, that is, the corresponding 9-aminoacridines and acridones.

The analytical procedures developed for the potential fluorescent labeling agents were also applied to the medicinally important 9-aminoacridine, quinacrine [6-chloro-2-methoxy-9-(1-methyl-4-diethylaminobutylamino)acridine] (I). As summarized in Scheme I, quinacrine (I) degrades to 6-chloro-2-methoxyacridone (II) in neutral (1, 2) or basic (3) conditions while 6-chloro-2-methoxy-9-aminoacridine (III) is formed under acidic conditions (3). The purpose of this report is to describe the application of sensitive chromatographic procedures for the examination of the purity of 9-acridine derivatives. The determination of quinacrine in the presence of its degradation products serves as the model system in this regard.

Scheme I—Hydrolysis of quinacrine as described in the literature

Table I—GLC Results

Compound	R_t, min.[a]	N[b]	Experimental[c] Conditions
Acridone	113.6	2039	I
9-Aminoacridine	12.8	1872	II
Acridone	14.4	1893	II
9-Aminoacridine	3.8	1975	III
Acridone	4.1	2439	III
6-Chloro-2-methoxy-9-aminoacridine	11.2	2584	III
6-Chloro-2-methoxy-9-phenoxyacridine	11.2	2212	III
6-Chloro-2-methoxyacridone	13.5	1938	III
Quinacrine	24.1	2560 (dec.)	III
9-(p-Aminobenzyl)acridine	23.7	2017	III
9-(p-N′-Benzyl-N-thioureido)-benzylacridine	41.5	2170	III
9-(p-Isothiocyanatobenzyl)-acridine	41.5	2120	III
6-Chloro-2-methoxy-9-(p-isothiocyanatobenzyl)-acridine	184	—[d]	III
9-Aminoacridine	12.1	2146 (tailing)	IV
9-Isothiocyanatoacridine	13.7	1655	IV
Acridone	13.8	1852 (tailing)	IV
9-Aminoacridine	1.5	—[d]	V
Acridone	1.6	—[d]	V
6-Chloro-2-methoxy-9-aminoacridine	4.1	1194 (tailing)	V
6-Chloro-2-methoxyacridone	4.2	1244 (tailing)	V
Quinacrine	12.7	2853	V

[a] R_t values are measured from solvent peak. [b] $N = 5.53(d/W_{0.5h})^2$, where d = retention time from injection point, and $W_{0.5h}$ = width at half height of peak in millimeters. [c] Experimental conditions: I, 2% Carbowax 20M on Gas Chrom Q [1.83-m. (6-ft.) × 4.0-mm. i.d. glass column], helium linear velocity ($\bar{U}_0$) = 11.0 cm. sec.$^{-1}$, column temperature 201°, injection temperature 209°, and detector temperature 202°; II, 5.2% OV-17 on Gas Chrom Q [1.83-m. (6-ft.) × 4.0-mm. i.d. glass column], $\bar{U}_0$ = 11.0 cm. sec.$^{-1}$, column temperature 211°, injection temperature 225°, and detector temperature 211°; III, 5.2% OV-17 on Gas Chrom Q [1.83-m. (6-ft.) × 4.0-mm. i.d. glass column], $\bar{U}_0$ = 10.4 cm. sec.$^{-1}$, column temperature 250°, injection temperature 270°, and detector temperature 260°; IV, 3.8% UCC-W98 on Diatoport S [1.23-m. (4-ft.) × 4.0-mm. i.d. glass column], $\bar{U}_0$ = 11.0 cm. sec.$^{-1}$, column temperature 182°, injection temperature 232°, and detector temperature 201°; and V, same conditions as IV with column temperature 200°, injection temperature 230°, and detector temperature 210°. [d] Not measurable.

EXPERIMENTAL[1]

GLC Systems—Two systems were employed: System A, 3.8% UCC-W98 on Diatoport S, 80–100, in a 1.23-m. (4-ft.) × 0.4-mm. i.d. glass column; and System B, 5.2% OV-17 on Gas Chrom Q, 80–100, in a 1.83-m. (6-ft.) × 0.4-mm. i.d. glass column. Operating conditions of temperatures and helium carrier gas flow are given in Table I. Standard solutions were prepared by dissolving 5 mg. of compounds

[1] All GLC was performed on a Hewlett-Packard 5750 chromatograph with flame-ionization detection (H_2 flow: 45 ml. min.$^{-1}$; air: 470 ml. min.$^{-1}$.

Table II—Relative R_f[a] Values with Different Solvent Systems

Compound	Relative R_f Values in Solvent Systems[b] I	II	III	IV	V	VI	VII	VIII	IX
Quinacrine hydrochloride	1.00(0.22)[c]	1.00(0.64)	1.00(0.45)	1.00(0.06)	1.00(0.05)	1.00(0.01)	1.00(0.46)	1.00(0.39)	1.00(0.39)
6-Chloro-2-methoxy-9-aminoacridine	2.77	1.20	1.55	6.3	4.4	8.0	0.87	0.97	0.97
6-Chloro-2-methoxy-acridone	3.25	1.31	1.69	9.3	8.8	35.0	1.17	1.23	1.23

[a] Relative R_f = R_f of compound/R_f of quinacrine hydrochloride. [b] Solvent systems by volume: I, methanol–28% ammonia (99:1); II, benzene–methanol–28% ammonia (74:74:2); III, benzene–methanol–28% ammonia (30:118:2); IV, benzene–ethyl acetate–28% ammonia (30:118:2); V, benzene–ethyl acetate–triethylamine (30:118:2); VI, benzene–ethyl acetate–triethylamine (70:74:2); VII, benzene–acetone–28% ammonia (74:74:2); VIII, cyclohexane–acetone–28% ammonia (74:74:2); and IX, *n*-heptane–acetone–28% ammonia (74:74:2). [c] The value in parentheses is the R_f value for quinacrine hydrochloride on a given chromatogram.

Table III—Fluorometric Determination on TLC Plates

Chromatogram Number	Compound	Wavelength Activation, nm.	Fluorescence, nm.	Meter Multiplier	Sensitivity	Amount Applied, ng.	Mean Fluorescent Intensity[a]	Mean Standard Deviation, %
1	Quinacrine hydrochloride	280	510	0.003	25.0	23.4, 46.8, 70.2, 93.6	425, 807, 1182, 1635	13.9
2	Quinacrine hydrochloride	285	500	0.003	25.0	23.4, 46.8, 70.2, 93.6, 117.0	254, 588, 880, 1199, 1561	11.2
3	6-Chloro-2-methoxy-9-aminoacridine	(a) 410[b] (b) 270	480 480	0.01 0.03	26.5 9.0	21.6, 43.2, 46.8, 86.4, 108.0	175, 512, 947, 1268, 1620 195, 524, 942, 1301, 1667	13.0 15.6
4	6-Chloro-2-methoxy-acridone	(a) 400[b] (b) 270	460 470	0.01 0.03	41.0 41.0	11.4, 22.8, 34.2, 45.6, 57.0	383, 649, 840, 1151, 1540 377, 588, 844, 1054, 1329	10.1 7.2
5	Mixtures					Mixture I, II, III, IV, V	Mixture I, II, III, IV, V	
	Quinacrine hydrochloride	280	500	0.003	29.0	99.4, 81.9, 64.3, 46.8, 29.2	1489, 1140, 1003, 779, 491	11.9
	6-Chloro-2-methoxy-9-aminoacridine	270	480	0.001	25.0	5.4, 10.8, 16.2, 21.6, 27.0	—[c] 260, 485, 892, 1352	13.4
	6-Chloro-2-methoxyacridone	270	480	0.001	38.0	5.7, 11.4, 17.1, 22.8, 28.5	392, 612, 923, 1368, 1753	11.8
6	Mixtures							
	Quinacrine hydrochloride	280	490	0.003	25.0	Same as Chromatogram 5 above	6298, 4434, 3564, 2525, 1977	15.7
	6-Chloro-2-methoxy-9-aminoacridine	270	490	0.001	24.0	—	—[c] 1154, 2264, 3243, 5592	15.1
	6-Chloro-2-methoxyacridone	270	480	0.001	33.0	—	1540, 2719, 3824, 4765, 6341	8.1

[a] Fluorescent intensity is expressed as peak area (height × width at half height) and is the average of seven spots at each concentration scanned in both directions. [b] There is less interference when activation is at 270 nm. [c] The peak at this concentration was too weak for accurate measurement.

in 0.5 ml. dimethyl sulfoxide plus 4.5 ml. of 95% ethanol, with 1-µl. volumes injected.

TLC—Initial separations by TLC were accomplished on 20 × 20-cm. 250-µ silica gel G[2] plates (30 g./100 ml. of water). The following were also used: quinacrine hydrochloride, 11.7 mg./10 ml. in 95% ethanol; 6-chloro-2-methoxy-9-aminoacridine, 4.9 mg./0.5 ml. in dimethyl sulfoxide plus 4.5 ml. 95% ethanol; 6-chloro-2-methoxyacridone, 5.8 mg./0.5 ml. in dimethyl sulfoxide plus 4.5 ml. 95% ethanol. Volumes of 1 µl. were applied. Nine solvent systems were evaluated (Table II). Based upon their fluorescent properties, compounds were detected by visualization under UV light.

For quantitative TLC fluorometric determinations, plates were scored into 0.5-cm. channels and spotted with 1-µl. volumes of solutions of quinacrine hydrochloride and its hydrolytic products in amounts as indicated in Table III. Each compound was spotted seven times at each concentration and chromatographed in System 7 [benzene–acetone–28% ammonia (74:74:2 v/v)]. Plates were air dried and measured with a thin-layer scanner[3] attached to a spectrophotofluorometer[4] and recorder[5]. Each spot was scanned both in the low to high R_f and high to low R_f directions under the conditions outlined in Table III. Fluorometric intensities were calculated from the recorded peaks, multiplying peak height by width at half height.

RESULTS AND DISCUSSION

Initially, GLC procedures were explored for both the assay of the parent 9-acridine compounds and their possible hydrolytic products. Preliminary GLC measurements were obtained on a 2% Carbowax 20M Gas Chrom Q column [1.83-m. (6-ft.) × 4.0-mm. i.d. glass]. However, as illustrated in Table I for acridone, retention times were excessive even at a column temperature of 200°, which approaches the thermal stability of the column. Measurements of lower molecular weight compounds and hydrolytic products were successful on a 5.2% OV-17 column (Table I). However, with increased molecular weight, retention on this column became excessive to irreversible, accompanied by indications of decomposition.

[2] Merck.
[3] Amino 4-8221A with scanner motor of 1 r.p.m.
[4] Aminco-Bowman 4-8106 with slit arrangement 4.
[5] Heath EU-20B.

A 3.8% UCC-W98 column was suitable for quantitative determination of higher molecular weight polyfunctional 9-acridine derivatives. For example, quinacrine hydrochloride had an R_t of 12.7 min. with a theoretical plate number (N) of 2853. This column was also suitable for the detection of the hydrolytic products of quinacrine (Compounds II and III). However, as indicated in Table I, these products have similar retention times, preventing the detection of one in the presence of the other at a column temperature of 200°. Note a decrease in the number of theoretical plates which, together with noticeable peak tailing, demonstrated a loss in column efficiency for these compounds. While excess tailing prevented the separation of Compounds I and II at lower column temperatures, this was possible for the lower molecular weight nonchlorinated parent compounds. Thus, while 9-aminoacridine could not be resolved from acridone at 200°, this could be accomplished at 182° (Table I).

Small amounts (100 ng.) of hydrolytic products could be detected in the presence of larger amounts (10 mcg.) of quinacrine hydrochloride on the 5.2% OV-17 column. On the 3.8% UCC-W98 column, the detection limit of quinacrine hydrochloride was about 100 ng. in the presence of excess (10 mcg.) hydrolytic products.

TLC fluorometric scanning was explored as an alternative method for the determination of quinacrine in the presence of its hydrolytic products. The development of suitable solvent systems is outlined in Table II. While chromatograms sprayed with 10% HCl followed by iodoplatinate reagent (4) visualized only the quinacrine as a weak blue-violet spot, compounds were readily detected by their natural fluorescence. The resolution of compounds was most satisfactory in Systems 3, 4, 5, 6, and 7, with mobility most satisfactory in Systems 1 and 7.

Of these two possibilities, System 7 was selected for quantitative TLC fluorometric scanning (Table III). Each compound was spotted at each concentration seven times, both as single compounds and as a mixture of compounds.

The mean of standard deviations for each compound determined separately and in mixture was 12.8%. The mean standard deviations for each chromatogram are listed in Table III. There was no significant difference in the standard deviations for a given compound for the determinations of compounds in mixture as compared to the determinations of single compounds. Factors responsible for the relatively high mean standard deviation include lack of uniformity of the thin-layer plates, the normal difficulty in reproducibility in plate spotting, and instrumental errors. In regard to plate uniformity, it should be noted that plates prepared in the laboratory were employed in this study in contrast to the more uniform commercially available plates.

In common with other *in situ* measurements of thin-layer plates (5, 6), it is not advisable to make comparisons between different plates nor to employ calibration curves. In general, linear relationships were obtained for calculated peak areas with respect to amounts applied. However, near the detection limits (5–10 ng.), there were deviations and the relationship did not always pass through the origin. Deviation from linearity owing to quenching was not observed for the maximum amounts (~100 ng.) used in this study.

Quantitative measurements are best made by multiple comparisons to known concentrations of standards on the same plate. It is important to emphasize that quantitative measurements could be made on a submicrogram level (10–100 ng.) with specificity provided simultaneously by TLC separation.

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Nature of Acetaminophen–Antipyrine Complex

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Abstract ☐ Acetaminophen and antipyrine form a single 1:1 complex which is shown to be stabilized through hydrogen bonding. IR spectroscopy shows both the —NH and —OH groups of acetaminophen and the carbonyl group of antipyrine to be involved in the complexation; the carbonyl group of acetaminophen is not. Methylation of either the —NH or the —OH group of acetaminophen is shown by phase equilibrium studies to prevent complexation with antipyrine.

Keyphrases ☐ Acetaminophen–antipyrine complex—nature of complexation ☐ Antipyrine–acetaminophen complex—nature of complexation ☐ Complexes—studies of complexation of acetaminophen–antipyrine

Antipyrine (phenazone) is known to complex with a wide variety of compounds and metallic ions (1); a number of workers (2–4) have reported complexation between antipyrine and phenols, with phenol itself forming a 1:1 complex with antipyrine (4). Complex formation has been reported not to occur between antipyrine and either acetanilide (5) or phenacetin (6), but a 1:1 complex of acetaminophen (*p*-acetamidophenol, *p*-hydroxyacetanilide) and antipyrine was recently patented (7) for its analgesic and antipyretic properties. This paper reports an investigation of the nature of that complex.